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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/710,058	11/10/2000	David Anderson	A-68531-1/RMS/JJD/SPL	4112

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EXAMINER	
LIU, SUE XU	

ART UNIT	PAPER NUMBER
1639	

DATE MAILED: 03/22/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/710,058	ANDERSON ET AL.	
	Examiner	Art Unit	
	Sue Liu	1639	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 February 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3 and 20-22* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3 and 20-22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

NOTE the change of examiner in this application.

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 2/17/2006 has been entered.

2. The amendment to the Claims and applicants' response filed on 2/17/06 have been fully considered and entered into the application.

3. Claims 1-3 and 20 are previously presented;

Claims 21 and 22 have been added;

Claims 1-3, and 20-22 are currently pending and are being examined in this application.

Priority

4. This application claims priority to U.S. Provisional Patent Application Nos. 60/164,592, filed 11/10/1999.

Specification

5. The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

Outstanding Objection (s) and/or Rejection (s)

Claim Rejections - 35 USC 103

1. Claims 1- 3 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bryan et al. US Pat. No. 6,232,107 (5/01: filed 10/98 or earlier) with attached Result 4 DATABASE Alignment search and Aran et al. Cancer Gene Therapy, Vol. 5, No. 4 pages 195-206 (1998).

The presently claimed invention is directed to:

Claim 1: A retroviral vector "for use in a mammalian cell" comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type *Renilla Mullerie* GFP).

Claim 2: The retroviral vector further comprises "a first encoding polynucleotide" and an "IRES site".

Claim 3: A "Cell" comprising a retroviral vector according to claim 1 or 2.

Claim 20: the use of "human codon-optimized nucleic acid encoding a Renilla GFP.

Initially it is noted that intended use language (e.g. "for use in a **mammalian** cell") , with respect to claims 1-3, with regard to cell type is not afforded patentable weight since:

a. Intended use language of compound/composition claims are normally not afforded patentable weight;

b. The specification encompasses both prokaryotic and eukaryotic host cells;

c. Doctrine of claim differentiation. Claim 3 broadly encompasses any cell type. Limiting claim 1 to mammalian cells would make claim 3 fail to further limit the scope of claim 1 and would be objectionable thereon.

However, with regard to claim 20, patentable weight is being afforded "for use in mammalian cell" in light of the use of "human codon-optimized nucleic acid encoding a Renilla GFP"; such codon-optimization being directed toward increasing expression in mammalian (e.g. human) cells.

Bryan et al. disclose and claim the use (e.g. diagnostics and high throughput screening e.g. libraries) of nucleic acid molecules encoding green fluorescent proteins (e.g. bioluminscent) from the genus Renilla, including nucleotide reference Seq. ID No. 15 which is 98.4% (with best local similarity of 99.4%) homologous to elected seq. ID 1; differing by only one nucleotide (C vs. G). Bryan further teaches protein Seq. Id. No. 16 which corresponds (e.g. has 100% sequence identity) to "wild type" Renilla GFP of Seq. Id. 2, as presently claimed [Compare . Reference Seq. Id 15 and attached Result 4 DATABASE Alignment search and Reference sequence Id. 16].

Bryan et al. further teach host cells (e.g. present claims 1 and 3) including prokaryotic/eukaryotic (e.g. mammalian) which incorporate genetic constructs comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type *Renilla Mullerie* GFP) as well as the use of "human codon-optimized nucleic acid encoding a Renilla GFP" as in present claim 20 (e.g. "The genes can be modified by substitution of codons optimized for expression in selected host cells or hosts, such as humans and other mammals ..." . See col. 5). The reference further teaches the use of a fusion partner (e.g. a targeting agent as a first gene) in its genetic fusion constructs as in present claims 2 and 3. See e.g. col. 8; col. 11-15; col. 24. Additionally, Bryan et al. teach the use of the bioluminescent green fluorescent proteins in cellular assays (e.g. live cells, including mammalian) and in high throughput screening systems (e.g. employing libraries) (e.g. see col. 2-3; 14).

It is important to note that the Bryan et al. reference, although teaching both (jellyfish) wild-type *Aequorea Victoria* GFP and (sea pansy) wild-type *Renilla Mullerie* GFP; the use of *Renilla* is strongly preferred due to the analytical problems present in the former. Particularly, *Aequorea* GFP possess two separate excitation peaks, whereas *Renilla* GFP has one excitation peak making it not ideal for use in analytical and diagonostic purposes:

> "Consequently, (jellyfish) GFP mutants have been selected with the hope of **identifying mutants** that have *single excitation spectral peaks* shifted to the red (emphasis provided). In fact **a stated purpose in constructing such mutants has been to attempt to make *A. Victoria* GFP more like the GFP from *Renilla*,** which has thus far not been cloned, but which has properties that make it far more ideal for use as an analytical tool. For many practical applications, the spectrum of Renilla GFP would be preferable to that of the Aequorea GFP, because *wavelength discrimination* between different fluorophores and detection of resonance energy transfer are easier if the component spectra are tall and narrow rather than low and broad". (emphasis provided)

See '107 col. 3-5; col. 47-48.

The Bryan et al. reference differs, if at all, from the presently claimed invention (e.g. see claims 1, 3 and 20) in failing to *explicitly teach* the use of a retrovirus as a vector.

However, in this regard, the Bryan et al. reference teaches that a wide variety of multipurpose vectors suitable for the expression of heterologous proteins are known to those of skill in the art and are commercially available; with selection and use of such vehicles as being well within the skill of the artisan. In this regard, the Bryan et al. vectors for use in mammalian hosts include "**recombinant *virus***" , as well as plasmid and phages e.g. the use of "**retroviral** long-terminal repeats and inducible promoters from other eukaryotic expression systems".. See e.g. col. 23 (especially bottom) to col. 24; col. 59-60 (emphasis provided). Accordingly, the Bryan et al. reference taken alone provides motivation to select the use of retroviral vectors, especially for use in mammalian host cells.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to select a retroviral vector for use in a cellular host (e.g. procarytoic or mammalian) with use of a genetic construct comprising a polynucleotide (e.g. cDNA) encoding a wild-type Renilla green fluorescent protein (GFP) or a fusion thereof with a reasonable expectation of success in light of the reference's ability to express Renilla GFP and in view of the benefits of using Renilla GFP (e.g. as compared to *A. Victoria* GFP.

To the extent that further motivation to select a retroviral vector is needed and to the extent that Bryan et al. fails to teach the incorporation of an IRES site (e.g. in present claim 2) in its fusion constructs, the Aran et al. reference is cited.

The Aran et al. reference teaches the favorable use of retroviral vectors, both in vitro and in vivo including an internal ribosome entry site (IRES) for fusion constructs preferably comprising optimized, humanized (e.g. see page 204, left column for benefits of humanizing) GFP (e.g. *Aequorea victoria*) ; since "[T]his vector allows rapid and specific identification of the expressed protein (e.g. MDR1 gene transfer) in living cells (e.g. mammalian cells) " (E.g. see Abstract and page 195, especially right column).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicants invention to utilize a retroviral vector as the Bryan et al. "recombinant virus" vector with the use of an IRES for expressing humanized or non-humanized wild-type renilla GFP in the Bryan et al. reference in order to appreciate the benefits thereof ; e.g. rapid and specific identification of the expressed protein.

2. Claim 20 is rejected under 35 U.S.C. 103(a) as being unpatentable over the obviousness rejections using Aran et al. And Bryan et al. as applied to claims 1-3 and 20 above, and, if necessary, further in view of Zolutukhin et al. US Pat. No. 5,874,304 (2/99: filed 1/96).

The above combined teaching of the Aran et al. and Bryan et al. References as described in the above obviousness rejection is hereby incorporated by reference in their entirety.

The combined reference teaching differs, if at all, from the presently claimed invention (e.g. claim 20) by failing to *explicitly* teach a human codon-optimized nucleic acid encoding a Renilla GFP (e.g. humanized GFP) in a retroviral vector.

However, Zolutukhin et al. teach that utilizing human codon-optimized nucleic acid GFP in nucleic acid constructs (including fusion proteins; e.g. col. 4 corresponding to present claim 2 "first gene" terminology) serves to overcome prior art obstacles and is advantageous (e.g. improved expression in mammalian and human cells). These constructs are included in vectors (e.g. see col. 5, examples; particularly retroviral: see patent claims, especially claims 50 and 69) contained in cells (e.g. see patent claims 71-80) in which the constructs contain:

1. GFP (particularly Renilla: see col. 1 , last paragraph; col. 14 and Table 1; and especially col. 16, lines 3-15: A... spectrum of Renilla ... preferable to that of Aequorea);

2. IRES elements (e.g. see col. 13; particularly patent claims 50 and 62).

See see col. 1-2).

Accordingly, one of ordinary skill in the art at the time of applicant's invention would have been motivated to utilize human codon-optimized nucleic acids expressing Renilla GFP in the genetic constructs (e.g. cells/vectors comprising renilla GFP/IRES elements) rendered obvious by the combined Aran et al. And Bryan et al. teaching in light of the advantages thereof imparted by such humanized sequences as taught by the Zolutukhin et al. reference.

Thus, it would have been prima facie obvious to one of ordinary skill at the time of applicant's invention to modify the cellular/vector genetic constructs taught by the Aran and Bryant reference to include human codon-optimized (e.g. humanized) nucleotides encoding renilla GFP in order to obtain the advantages thereof as taught by the Zolutukhin et al. reference.

3. Claims 1-3 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zolutukhin et al. US Pat. No. 5,874,304 (2/99: filed 1/96) and Bryan et al. US Pat. No. 6,232,107 (5/01: filed 10/98 or earlier)with attached Result 4 DATABASE Alignment search.

The presently claimed invention is directed to:

Claim 1: A retroviral vector "for use in a mammalian cell" comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type *Renilla Mullerie* GFP).

Claim 2: The retroviral vector further comprises "a first encoding polynucleotide" and an "IRES site" .

Claim 3: A "Cell" comprising a retroviral vector according to claim 1 or 2.

Claim 20: the use of "human codon-optimized nucleic acid encoding a Renilla GFP.

Initially it is noted that intended use language (e.g. "for use in a **mammalian** cell") , with respect to claims 1-3, with regard to cell type is not afforded patentable weight since:

a. Intended use language of compound/composition claims are normally not afforded patentable weight;

b. The specification encompasses both prokaryotic and eukaryotic host cells;

c. Doctrine of claim differentiation. Claim 3 broadly encompasses any cell type. Limiting claim 1 to mammalian cells would make claim 3 fail to further limit the scope of claim 1 and would be objectionable thereon.

However, with regard to claim 20, patentable weight is being afforded "for use in mammalian cell" in light of the use of "human codon-optimized nucleic acid encoding a Renilla GFP"; such codon-optimization being directed toward increasing expression in mammalian (e.g. human cells).

The Zolutukhin et al. reference teaches that utilizing human codon-optimized nucleic acid GFP in nucleic acid constructs (including fusion proteins; e.g. col. 4 corresponding to present claim 2 "first gene" terminology) serves to overcome prior art obstacles and is advantageous (e.g. improved expression in mammalian and human cells). These constructs are included in vectors (e.g. see col. 5, examples; particularly retroviral: see patent claims, especially claims 50 and 69) contained in cells (e.g. see patent claims 71-80) in which the constructs contain:

1. GFP (particularly Renilla: see col. 1 , last paragraph; col. 14 and Table 1; and especially col. 16, lines 3-15: A... spectrum of Renilla ... preferable to that of Aquorea);

2. IRES elements (e.g. see col. 13; particularly patent claims 50 and 62. (e.g. see col. 1-2).

It is noteworthy that Zolutukhin teaches that (sea pansy) Renilla GFP is more preferable as a reporter than Aequorea GFP since Aequorea has two absorbance peaks whereas Renilla GFP has a single absorbance peak at 498 accordingly:

For many practical applications, the spectrum of Renilla GFP would be preferable to that of Aequorea because wavelength discrimination between different fluorophores and detection of resonance energy transfer are easier when the component spectra are tall and narrow rather than low and broad." Accordingly, Zolutukhin (like the Bryan reference) teaches mutation of Aequorea toward obtaining a single peak (e.g. like Renilla) is desired. See Zolutukhin at col. 16.

Although the Zolutukhin et al. reference teaches nucleic acid which employ the preferential use of Renilla GFP, the Zolutukhin reference differs from the presently claimed invention by failing to explicitly teach the use of a *Renilla* GFP gene sequence which encodes wild type Renilla GFP corresponding to SEQ Id. 2.

Bryan et al. disclose and claim the use (e.g. diagnostics and high throughput screening e.g. libraries) of nucleic acid molecules encoding green fluorescent proteins (e.g. bioluminscent) from the genus Renilla, including nucleotide reference Seq. ID No. 15 which is 98.4% (with best local similarity of 99.4%) homologous to elected seq. ID 1; differing by only one nucleotide (C vs. G). Bryan further teaches protein Seq. Id. No. 16 which corresponds (e.g. has 100% sequence identity) to "wild type" Renilla GFP of Seq. Id. 2, as presently claimed [Compare . Reference Seq. Id 15 and attached Result 4 DATABASE Alignment search and Reference sequence Id. 16].

Bryan et al. further teach host cells (e.g. present claims 1 and 3) including prokaryotic/eukaryotic (e.g. mammalian) which incorporate genetic constructs comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type *Renilla Mullerie* GFP) as well as the use of "human codon-optimized nucleic acid encoding a Renilla GFP" as in present claim 20 (e.g. "The genes can be modified by substitution of codons optimized for expression in selected host cells or hosts, such as humans and other mammals ..." . See col. 5). The reference further teaches the use of a fusion partner (e.g. a targeting agent as a first gene) in its genetic fusion constructs as in present claims 2 and 3. See e.g. col. 8; col. 11-15; col. 24.

Bryan et al. teach the use of the bioluminescent green fluorescent proteins in cellular assays (e.g. live cells, including mammalian) and in high throughput screening systems (e.g. employing libraries) (e.g. see col. 2-3; 14).

It is important to note that the Bryan et al. reference, **like the Zolutukhin et al. reference**, although teaching both (jellyfish) *Aequorea Victoria* GFP and (sea pansy) wild-type *Renilla Mullerie* GFP; teach that the use of *Renilla* is strongly preferred due to the analytical problems present in the former. Particularly, *Aequorea* GFP possess two separate excitation peaks, whereas *Renilla* GFP has one excitation peak making it not ideal for use in analytical and diagonostic purposes:

> "Consequently, (jellyfish) GFP mutants have been selected with the hope of **identifying mutants** that have *single excitation spectral peaks* shifted to the red (emphasis provided). In fact a stated purpose in constructing such mutants has been to attempt to make *A. Victoria* GFP more like the GFP from *Renilla*, which has thus far not been cloned, but which has properties that make it far more ideal for use as an analytical tool. For many practical applications, the spectrum of Renilla GFP would be preferable to that of the Aequorea GFP, because *wavelength discrimination* between different fluorophores and detection of resonance energy transfer are easier if the component spectra are tall and narrow rather than low and broad". (emphasis provided)

See '107 col. 3-5; col. 47-48.

Thus, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to utilize the Bryan et al. polynucleotide Renilla green fluorescent protein (including seq. Id 15) in the Zolutukhin reference genetic constructs since:

a. BOTH Zolutukin and Bryan et al. teach the preferential use of Renilla GFP thus motivating the selection of the Bryan Renilla GFP obvious to one of ordinary skill in the art; and/or

b. one of ordinary skill in the art would have been motivated to select the Bryan reference Renilla sequences for purposes of performing screening assays (e.g. high throughput library screens) in order to obtain the benefits of the renilla protein in such assays as taught by the Bryan reference.

Discussion

10. Applicant's arguments filed on 2/17/06 regarding the art rejections of claims over combined teachings of Bryan et al, and DATABASE Alignment search and Aran et al and Zoulutukhin et al have been fully considered but they are not persuasive.

11. Applicants traversal of all the rejection has been considered and is not persuasive for the following reasons.

Claim Interpretation To clarify the confusion over the interpretation of the instant claims (especially Claims 1 and 20), the claims are interpreted as the followings:

The instant claims are drawn to a retroviral vector comprising a polynucleotide, which encodes for a GFP having a specific amino acid sequence (Seq ID NO2). The specific amino acid sequence recited in SEQ ID NO2 is an exact match to the wildtype amino acid sequence for a Renilla GFP. The instant claims (Claim 20) further limit the said polynucleotide to comprise a human codon-optimized nucleic acid encoding a Renilla GFP. That is the coding nucleic acid sequences for the said GFP protein (with amino acid sequence recited in SEQ ID NO2) is humanized (or mutated from the wildtype DNA sequences to possess human codons).

Traversal Over Prior Art In applicants' arguments, applicants traversed over the cited prior art for the 103 rejections as set forth in previous office actions. The main traversal is that the result of the instant application is unexpected, and that the prior art cited teach away from the claimed invention. Therefore, there would be no motivation to combine the teachings of the cited 103 references. Specifically, applicants have cited the Hanazona, the Cheng, and the Levy

references to show that a retroviral vector comprising a wild-type GFP would not work (i.e. no detectable fluorescence in a retroviral-mammalian gene expression system).

First, the above traversal is irrelevant to argue against the 103 rejections over claims directed to a "retroviral vector", which is essentially a polynucleotide vector that comprises various elements (nucleic acid sequences). The question of whether the inserted GFP gene expresses or not is not relevant for the discussion concerning the vector itself. The prior art (e.g. Aran et al) had shown that a "retroviral vector" (a polynucleotide sequence) comprising a GFP was made and verified. The prior cited 103 references also showed the it is well known in the art that one can insert different sequences in such vectors. Since Bryan et al teach the sequence of a Renilla GFP and its insertion in different vectors, it would have been obvious to one ordinary skilled in the art to generate a "retroviral vector" comprising that specific GFP gene sequence regardless whether the vector when transfected into mammalian cell would express or not. There would be reasonable expectation to achieve such a vector (i.e. a DNA construct comprising the GFP sequence) because the technique for generating such a vector is known and routine in the art as taught by the prior art. Since Applicants have not shown how the claimed retroviral vector is structurally and/or functionally different from the prior art, or how the vector construct is non-obvious over the prior art teachings, the 103 rejections against at least Claims 1, 2 and 20 are maintained.

Second, applicants argue that the prior art teach that wild-type GFP can not be expressed in a retroviral-mammalian gene expression system citing several references indicating the fact. Applicants argue that the references (Hanazona, Cheng, and Levy) teach that fluorescence was

undetectable from mammalian cells comprising GFP retroviral constructs. In order to answer applicants' argument, the following analysis of the cited references are provided:

Overall, all the references cited by the applicants to argue for unexpected results are drawn to *Aequoria* GFP but not *Renilla* GFP as discussed in previous office actions. Applicants argue that Aequoria wildtype GFPs cannot be expressed in a retroviral-mammalian gene expression system. However, applicants' claim to unexpected result is for the *Renilla* wildtype GFP. The state of the art at the time of the invention was made does not teach that Renilla wildtype GFP is incompatible with the retroviral mammalian gene expression system. Therefore, the cited references do not provide support for the unexpected results claimed for Renilla wildtype GFP since the GFPs are from two different species. Contrary to applicants' assertion, this view point is not in conflict with the 103 art rejection set forth by the previous office action. The art rejection is based on a 103 obviousness rejection, where the Bryan et al reference teaches that the wild-type Renilla GFP is strongly preferred over the Aequorea GFP due to the analytical problems presented in the latter (see page 4 of this office action). This provides strong motivation for one of skilled in the art at the time of the invention was made to use Renilla GFP instead of Aequorea GFP in a retroviral-mammalian gene expression system.

Hanazona The reference teaches construction of a retroviral vector for the mammalian expressing of **Mutant GFPs**. Applicants have maintained and stressed that the claimed SEQ ID No2 is drawn to the **wild-type** Renilla GFP and the cited references have shown that the wild-type GFP is incompatible with the retroviral-mammalian gene expression system, and therefore the 103 rejections can be overcame because there are unexpected results from the instant invention. Since the Hanazona reference only teaches a mutant form of GFP, it is irrelevant to

applicants' argument of unexpected results. Furthermore, although the reference does teach that no stable cell lines expressing GFP were produced, the reference teaches that a retroviral vector comprising a GFP (mutant form) was successfully produced, and the GFP containing retroviral vector was successfully introduced into mammalian cells (Page 1316, 1st paragraph). The reference teaches that "fluorescence of cells transfected with the GFP-containing plasmids was observed..." (Page 1316, 1st paragraph, line 2), which shows that the GFP containing viral vector was successfully introduced into the mammalian cells and GFP was indeed expressed regardless how long the transfected cell lived.

Levy The reference teaches constructing retroviral vector comprising different forms (wildtype and mutant) GFPs and transduction into mammalian cells. The "wildtype" GFP taught by the reference refers to "wildtype" both in terms of amino acid sequence and nucleic acid sequence. For example, the reference teaches that the "wildtype GFP gene without red-shift mutation or codon modifications" (emphasis added) exhibited little (when transiently transfected) or no (stable transfection) fluorescence (See the Table 1 and the Caption). The reference further teaches that with "humanization" (human codon usage) and a single amino acid mutation, the GFP was successfully expressed and intense fluorescence was observed. The conclusion quoted by applicants stating that "wildtype GFP could never be visualized" (p613, 1st paragraph) is referring to a wildtype GFP both in its coding sequence and amino acid sequence. The reference also teaches that several studies have discovered that by humanize the wildtype codons (pg 610, right col. 2nd paragraph), wildtype GFP (in term of protein sequence) can be successfully expressed in mammalian cells. The conclusion of this reference does not particularly indicate that wildtype (in term of amino acid sequence) is incompatible with

retroviral-mammalian expression system. The reference, however, does provide motivation to utilize humanized GFP coding nucleic acid sequence for construction of a retroviral vector to be used in a mammalian gene expression system.

Cheng The reference teaches expressing various forms of GFP in mammalian cells either through different expression vectors (transient and retroviral vectors). Similar to the Hanazono reference, the Cheng reference teaches retroviral vector comprising mutant form (in term of amino acid sequence) that was transduced into mammalian cells (See Figure 2 and Pg607, left col., 2nd paragraph). The reference teaches that the wildtype GFP gene (nucleic acid sequence) was used to construct a transient mammalian expression vector (not a retroviral vector) (See pg 606, right col., last paragraph). The reference further teaches that the wildtype GFP gene expressed in the transfected mammalian cells (See Figure 1) as analyzed by FACS. Contrary to applicants' interpretation, the reference does not teach that the wildtype GFP is incompatible with mammalian gene expression system, but the wildtype and mutant GFP "are stable and properly processed to form functional fluorophores." (Pg 608, right col., lines 1-5 under Discussion). The reference further teaches that the "Expression of GFPs, either transiently or stably, are not detrimental to host cells."

Therefore, the references cited by the applicants do not provide support for applicants' argument of unexpected result. These references, however, do provide ample evidence to show that various forms of GFP (including both wildtype and mutant forms) can be successfully inserted into a retroviral vector and expressed in mammalian cells. These references also provide motivations to humanize (alter wildtype GFP gene to have human codons) wildtype GFP gene for expression in mammalian cells. These reference at best have demonstrated that

expressing GFPs in mammalian cells (using various expression vectors such as retroviral vector) is highly feasible and successful, which would provide motivations for one skilled in the art to generate mammalian gene expression system with different GFPs (derived from different sources or mutant forms).

Therefore, without demonstrating how the claimed retroviral vector and mammalian cells are structurally and/or functionally different from or non-obvious over the claimed prior art teachings, the 103 rejections set forth in the previous office actions are still maintained for the reasons of record as well as the reasons set forth above.

New Rejections

Claim Rejections - 35 USC § 112

6. Claim 22 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

In the new Claim 22, the added intended use of screening a "test agent" with the recited mammalian cells does not appear to have support in the specification. Specifically, the instant specification does not provide support for the claimed "test agent".

If applicants disagree, applicant should present a detailed analysis as to why the claimed subject matter has clear support in the specification.

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Claims 1, 3, and 20-22 are rejected under 35 U.S.C. 103(a) as being obvious over Bierhuizen et al (Biochemical and Biophysical Research Communications. Vol. 234: 371-375; 1997), in view of Bryan et al (US Patent 6,232,107; 2001; Filed 3/26/1999; priority date: 3/27/1998).

The instant claims briefly recite a product of expression system comprising a mammalian cell and a retroviral vector comprising a GFP with an amino acid sequence comprising SEQ ID NO 2. The said mammalian cell has intended use of detected by FACS and testing for a test agent. These intended uses do not provide additional structural and/or functional limitations on the claimed mammalian cells.

Bierhuizen et al teach a using retroviral vector to transfer Green Fluorescent Protein (GFP) into mammalian cells (see Abstract of the reference). The reference teaches both wildtype and mutant (in term of amino acid sequence) of *Aequorea Victoria* GFP were expressed in mammalian cells (see Figure 1; page 373, left col., 1st paragraph; and page 374, left col., 1st paragraph). The reference also teaches that retroviral vectors comprising various GFP constructs were generated (See page 373, left col., 1st paragraph), which would read on a retroviral vector comprising a polynucleotide encoding a green fluorescent protein. In addition, the reference teaches that FACS was used to analyze GFP expression (See Figure 1 and caption). The

reference further teaches that humanized (meaning replacing Aequorea Victoria codons with human codons in the coding DNA sequence) GFP can achieve higher expression in mammalian cells (Page 371, right col., last lines of 2nd paragraph). Furthermore, the reference teaches that the purpose of the study was to evaluate the potential applicability of GFP expression as a marker for the rapid selection of retrovirally transduced mammalian cells (See page 374, left col., 1st line). The study of the reference conclusively teaches that the data showed that all variants (including the wildtype GFP) allow for flow cytometric detection (FACS) of stable GFP expression in mammalian cells and that he expression can be transferred by the MFG retroviral vector (See page 374, left col., 1st paragraph, last lines).

Bierhuizen et al do not specifically teach the Retinilla GFP with the specific amino acid sequence recited in SEQ ID No 2.

However, Bryan et al teach the use of Renilla GFP as described in previous office actions and is incorporated by reference to its entirety as set forth below. The reference teaches use (e.g. diagnostics and high throughput screening e.g. libraries) of nucleic acid molecules encoding green fluorescent proteins (e.g. bioluminscent) from the genus Renilla, including Seq. Id. No. 16 which corresponds (e.g. has 100% sequence identity) to "wild type" Renilla GFP of Seq. Id. 2, as presently claimed. Bryan et al. further teach host cells (e.g. present claims 1 and 3) including prokaryotic/eukaryotic (e.g. mammalian) which incorporate genetic constructs comprising a polynucleotide (e.g. cDNA) encoding a green fluorescent protein (GFP) having the amino acid sequence of SEQ ID 2 (e.g. (sea pansy) wild-type Renilla Mullerie GFP) as well as the use of "human codon-optimized nucleic acid encoding a Renilla GFP" as in present claim 20 (e.g. "The genes can be modified by substitution of codons optimized for expression in selected host

cells or hosts, such as humans and other mammals ..." . See col. 5). The reference further teaches the use of a fusion partner (e.g. a targeting agent as a first gene) in its genetic fusion constructs as in present claims 2 and 3. See e.g. col. 8; col. 11-15; col. 24. Additionally, Bryan et al. teach the use of the bioluminescent green fluorescent proteins in cellular assays (e.g. live cells, including mammalian) and in high throughput screening systems (e.g. employing libraries) (e.g. see col. 2-3; 14).

It is important to note that the Bryan et al. reference, although teaching both (jellyfish) wild-type *Aequorea Victoria* GFP and (sea pansy) wild-type *Renilla Mullerie* GFP; the use of *Renilla* is strongly preferred due to the analytical problems present in the former. Particularly, *Aequorea* GFP possess two separate excitation peaks, whereas *Renilla* GFP has one excitation peak making it not ideal for use in analytical and diagonostic purposes:

> "Consequently, (jellyfish) GFP mutants have been selected with the hope of **identifying mutants** that have *single excitation spectral peaks* shifted to the red (emphasis provided). In fact **a stated purpose in constructing such mutants has been to attempt to make *A. Victoria* GFP more like the GFP from *Renilla*,** which has thus far not been cloned, but which has properties that make it far more ideal for use as an analytical tool. For many practical applications, the spectrum of Renilla GFP would be preferable to that of the Aequorea GFP, because *wavelength discrimination* between different fluorophores and detection of resonance energy transfer are easier if the component spectra are tall and narrow rather than low and broad". (emphasis provided)

See '107 col. 3-5; col. 47-48.

The Bryan et al. reference differs, if at all, from the presently claimed invention (e.g. see claims 1, 3 and 20) in failing to *explicitly teach* the use of a retrovirus as a vector.

However, in this regard, the Bryan et al. reference teaches that a wide variety of multipurpose vectors suitable for the expression of heterologous proteins are known to those of skill in the art and are commercially available; with selection and use of such vehicles as being

well within the skill of the artisan. In this regard, the Bryan et al. vectors for use in mammalian hosts include "**recombinant *virus***", as well as plasmid and phages e.g. the use of "**retroviral** long-terminal repeats and inducible promoters from other eukaryotic expression systems".. See e.g. col. 23 (especially bottom) to col. 24; col. 59-60 (emphasis provided). Accordingly, the Bryan et al. reference taken alone provides motivation to select the use of retroviral vectors, especially for use in mammalian host cells.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to select a retroviral vector for use in a cellular host (e.g. procarytoic or mammalian) with use of a genetic construct comprising a polynucleotide (e.g. cDNA) encoding a wild-type Renilla green fluorescent protein (GFP) or a fusion thereof with a reasonable expectation of success in light of the reference's ability to express Renilla GFP and in view of the benefits of using Renilla GFP (e.g. as compared to *A. Victoria* GFP).

Therefore, it would have been prima facie obvious for an ordinary skilled artisan to screen for generate a retroviral vector comprising a polynucleotide encoding a GFP with a specific amino acid sequence (e.g. wildtype Renilla GFP amino acid sequence). Due to the advantages taught by both Bierhuizen et al and Bryan et al that GFP allows rapid selection of retrovirally transduced mammalian cells, a person of ordinary skill in the art would have been motivated at the time of the invention to construct a retroviral vector comprising a specific GFP (e.g. Renilla GFP) for using in a mammalian gene expression system. Since the construction of retroviral vector comprising various GFPs (including wildtype, mutant, or humanized) is known in the art (such as taught by Bierhuizen et al), and the specific sequence of a Renilla GFP is known and expressable in mammalian cells (as taught by Bryan et al), an ordinary skilled artisan

would have been motivated to generate a retroviral vector comprising GFP having a specific amino acid sequence and a mammalian cell comprising the retroviral vector. An ordinary skilled artisan would have reasonable expectation of success of achieving such modifications since Bierhuizen et al have demonstrated the success of generating retroviral vector comprising GFP used in mammalian cell expression.

In conclusion, the invention of the instant claims would have been prima facie obvious over Bierhuizen et al, in view of Bryan et al to one of ordinary skill in the art without evidence to the contrary.

9. Claims 1-3, and 20-22 are rejected under 35 U.S.C. 103(a) as being obvious over Bierhuizen et al (Biochemical and Biophysical Research Communications. Vol. 234: 371-375; 1997), in view of Bryan et al (US Patent 6,232,107; 2001; Filed 3/26/1999; priority date: 3/27/1998) and further in view of Aran et al (Cancer Gene Therapy. Vol. 5: 195-206; 1998).

The instant claims briefly recite a product of expression system comprising a mammalian cell and a retroviral vector comprising a GFP with an amino acid sequence comprising SEQ ID NO 2. The said mammalian cell has intended use of detected by FACS and testing fro a test agent. These intended uses do not provide additional structural and/or functional limitations on the claimed mammalian cells.

Bierhuizen et al teaches a GFP retroviral vector and mammalian gene expression system as described supra.

Bryan et al teaches a Renilla GFP and its uses in various gene expression system as described supra.

Both of the references do not specifically teach the expression vector comprises IRES.

However, Aran et al teaches a retroviral vector comprising an IRES element (See Page 197, left col., 2nd paragraph). The reference also teaches that the retroviral vector comprises a GFP and is used to transducer a mammalian cell (page 197, left col., 2nd and last paragraphs). The reference further teaches the advantage of including IRES element such as the element allows efficient cap-independent translation of the downstream gene.

Therefore, it would have been prima facie obvious for an ordinary skilled artisan to screen for generate a retroviral vector comprising a polynucleotide encoding a GFP with a specific amino acid sequence (e.g. wildtype Renilla GFP amino acid sequence) and an IRES element. Due to the advantages taught by Aran et al that the inclusion of an IRES element in a retroviral vector expression system would facilitate gene expression, an ordinary skilled artisan would be motivated at the time of the invention to generate a retroviral GFP expression vector comprising an IRES element. In addition, the inclusion of an IRES element in a eukaryotic gene expression system is well known in art such as taught by Aran et al. An ordinary skilled artisan would have reasonable expectation of success of achieving such modifications since Aran et al have demonstrated the success of generating retroviral vector comprising GFP and IRES used in mammalian cell expression system.

In conclusion, the invention of the instant claims would have been prima facie obvious over Bierhuizen et al, in view of Bryan et al and further in view of Aran et al to one of ordinary skill in the art without evidence to the contrary.

10. Claims 1, 3, and 20-22 are rejected under 35 U.S.C. 103(a) as being obvious over Anderson et al (PNAS. Vol. 93: 8508-8511; 1996), in view of Bryan et al (US Patent 6,232,107; 2001; Filed 3/26/1999; priority date: 3/27/1998).

The instant claims briefly recite a product of expression system comprising a mammalian cell and a retroviral vector comprising a GFP with an amino acid sequence comprising SEQ ID NO 2. The said mammalian cell has intended use of detected by FACS and testing for a test agent. These intended uses do not provide additional structural and/or functional limitations on the claimed mammalian cells.

Anderson et al teach using retroviral vector expressing a GFP in mammalian cells. The reference teaches that retroviral gene transfer was used to stably incorporate the wildtype GFP in a mammalian cell (see page 8509, left col., 1st para. under RESULTS) and expressed in mammalian cells (NIH 3T3). The reference also teaches that FACS analysis of the retroviral vector comprising the wildtype GFP transduced cells revealed a single peak on a fluorescence histogram, and there was a two fold difference in fluorescence value between infected and uninfected cells (page 8509, left col., 1st para. under RESULTS). These would read on the fluorescence of the GFP can be detected by FACS since the fluorescence of the retroviral transduced cells in the reference was detected.

Anderson et al do not specifically teach that the cDNA for the GFP is humanized, and the specific GFP amino acid sequence.

However, Bryan et al teaches a Renilla GFP (with specific amino acid sequence) and its uses in various gene expression systems as described supra.

Therefore, it would have been prima facie obvious for an ordinary skilled artisan to screen for generate a retroviral vector comprising a polynucleotide encoding a GFP with a specific amino acid sequence (e.g. wildtype Renilla GFP amino acid sequence) that is encoded by a humanized cDNA. Due to the advantages taught by Bryan et al that GFP allows rapid selection of retrovirally transduced mammalian cells and the motivation to use GFP in mammalian expression system as discussed supra, a person of ordinary skill in the art would have been motivated at the time of the invention to construct a retroviral vector comprising a specific GFP (e.g. a humanized Renilla GFP) for using in a mammalian gene expression system. Since the construction of retroviral vector comprising various GFPs (including wildtype, mutant, or humanized) is known in the art (such as taught by Anderson et al), and the specific sequence of a Renilla GFP is known and is shown to be expressed in mammalian cells (as taught by Bryan et al), an ordinary skilled artisan would have been motivated to generate a retroviral vector comprising GFP having a specific amino acid sequence and a mammalian cell comprising the retroviral vector. An ordinary skilled artisan would have reasonable expectation of success of achieving such modifications since Anderson et al have demonstrated the success of generating retroviral vector comprising GFP used in mammalian cell expression.

In conclusion, the invention of the instant claims would have been prima facie obvious over Anderson et al, in view of Bryan et al to one of ordinary skill in the art without evidence to the contrary.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sue Liu whose telephone number is 571-272-5539. The examiner can normally be reached on M-F 9am-3pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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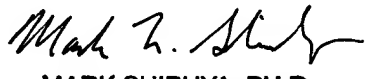

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